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Biodegradation of Petroleum Hydrocarbons by Psychrotrophic *Pseudomonas* Strains Possessing Both Alkane (*alk*) and Naphthalene (*nah*) Catabolic Pathways†

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Three hydrocarbon-degrading psychrotrophic bacteria were isolated from petroleum-contaminated Arctic soils and characterized. Two of the strains, identified as *Pseudomonas* spp., degraded C_5 to C_{12} *n*-alkanes, toluene, and naphthalene at both 5 and 25°C and possessed both the *alk* catabolic pathway for alkane biodegradation and the *nah* catabolic pathway for polynuclear aromatic hydrocarbon biodegradation. One of these strains contained both a plasmid slightly smaller than the *P. oleovorans* OCT plasmid, which hybridized to an *alkB* gene probe, and a NAH plasmid similar to NAH7, demonstrating that both catabolic pathways, located on separate plasmids, can naturally coexist in the same bacterium.

Microorganisms which biodegrade the various components of petroleum hydrocarbons such as polynuclear aromatic hydrocarbons (PAHs), including naphthalene, monoaromatic hydrocarbons such as toluene, or aliphatic hydrocarbons such as the *n*-alkanes, are readily isolated from the environment, particularly from petroleum-contaminated sites. Some of the microbial catabolic pathways responsible for the degradation, including the *alk* (C_5 to C_{12} *n*-alkanes), *nah* (naphthalene), and *xyl* (toluene) pathways, have been extensively characterized and are generally located on large catabolic plasmids usually found in *Pseudomonas* spp., for example, the OCT, NAH, and TOL plasmids (19). However, there are very few reports describing and characterizing microorganisms that can catabolize both aliphatic compounds and PAHs and that possess both the *alk* and *nah* catabolic pathways. For example, many of the 200 environmental strains isolated by Foght et al. (7) could mineralize aliphatic or aromatic hydrocarbons but not both, suggesting that alkane and PAH biodegradation may be mutually exclusive properties in bacteria. On the other hand, 36% of hydrocarbon-degradative strains isolated from sediments contaminated with oil from the 1989 *Exxon Valdez* spill had both *alkB* (encoding alkane monooxygenase [23]) and *xylE* (encoding catechol 2,3-dioxygenase [18]) genes, indicating that the *alk* and *xyl* pathways for aliphatic and aromatic hydrocarbon degradation can exist in the same organism (21). In addition, exconjugants of *Pseudomonas*, produced from laboratory matings of strains with OCT-CAM and NAH plasmids, displayed the Oct^+ and Nah^+ phenotypes (8). Several environmental isolates such as *Acinetobacter calcoaceticus* and *Alcaligenes odorans* (16), *Arthrobacter* sp. (4), and two *Rhodococcus* strains (17) were found to degrade both alkanes and naphthalene, although the genes and catabolic pathways responsible were not described.

In an initial investigation (2), PCR analysis of total-community DNA extracted from petroleum hydrocarbon-contaminated soils from the high Arctic indicated that some of the contaminated soils contained microorganisms possessing catabolic genes similar to *alkB* and *ndoB* (this gene and the highly homologous *nahAc* [20] and *pahAc* [22] encode the ISP_{NAP} subunit of naphthalene dioxygenase [14]). In the present study, a psychrotrophic strain isolated from the contaminated soil possessed two large plasmids, the smaller of which hybridized to an *ndoB* gene probe and the larger of which hybridized to an *alkB* gene probe. This is the first report of both the *alk* and the *nah* catabolic pathways contained on two different plasmids coexisting in a naturally occurring bacterium.

Isolation and characterization of psychrotrophic hydrocarbon biodegradative strains. The psychrotrophic strains used in this study were isolated from a petroleum-contaminated soil (coarse, granular) from Pangnirtung, Baffin Island, Northwest Territories, Canada. Viable bacterial counts in these soils averaged 10^4 to 10^5 CFU/g of soil. Isolated colonies grown at 5°C were transferred to MSM-YTS (10), checked for purity, and screened for the presence of naphthalene dioxygenase by the indigo test (5). Three indigo-positive strains designated BI7, BI8, and BI9 were isolated and identified by substrate utilization (Biolog GP microplate identification system; Biolog, Hayward, Calif.), cell wall fatty acid analysis (Microcheck Inc.), and 16S rDNA sequencing (essentially as described by Wang et al. [24]). The three strains were gram-negative, oxidase-positive, catalase-positive rods and produced nonpigmented, beige, circular colonies on tryptic soy agar (TSA). The three isolates were psychrotrophs, with growth temperature ranges of 0 to ca. 30°C, with optimum growth temperatures of ca. 25°C. Preliminary identification of BI7, BI8, and BI9 by the Biolog GP microplate identification system and by fatty acid composition indicated that they belonged to the genus *Pseudomonas*. 16S rDNA sequence analysis of BI7 and BI8 showed 96.4 and 96.8% DNA sequence identity, respectively, to *Pseudomonas agarici*. The 16S rDNA sequences of BI7 and BI8 were very similar but not identical (98.5% DNA sequence identity). On the basis of these data, these two strains are referred to as *Pseudomonas* sp. strain BI7 and *Pseudomonas* sp. BI8.

Nucleotide sequence accession numbers. The BI7 and BI8 16S rDNA sequences have been submitted to GenBank and assigned accession no. U81870 and U81871, respectively.

Screening of psychrotrophic isolates for mineralization activity and alkane substrate utilization. The ability of the psychrotrophic *Pseudomonas* spp. to mineralize ^{14}C-labelled hydrocarbon substrates at 5 and 25°C was determined as pre-

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TABLE 1. Utilization of petroleum hydrocarbon components by *Pseudomonas* sp. strains BI7 and BI8 at 25°C

Substrate	% of ^{14}C recovered as CO_2 in[a]:			Amt of CO_2 (μmol) evolved in[b]:	
	BI7	BI8	Uninoculated control	BI7	BI8
Mineralization					
Dodecane	17.4	15.6	0.5		
Hexadecane	5.3	5.1	0.2		
Naphthalene	70	75	0.4		
Fluorene	0.7	0.9	0.4		
Benzene	2.8	3.1	2.8		
Toluene	5.2	6.7	1.8		
Growth as sole C source					
No carbon source				0.5 (0.01)	8.5 (0.4)
Pentane				31.7 (5.6)	22.8 (3.1)
Hexane				23.8 (3.9)	48.3 (4.7)
Heptane				17.5 (2.1)	78.4 (7.2)
Octane				43.9 (1.0)	53.4 (7.4)
Decane				3.2 (0.1)	6.2 (0.2)
Undecane				2.1 (0.2)	24.0 (4.0)
Dodecane				2.6 (0.1)	15.0 (0.5)
Hexadecane				5.8 (0.3)	5.9 (0.5)

[a] Values represent the average percentage of ^{14}C recovered as CO_2 from duplicate samples after 2 weeks of incubation at 25°C in MSM supplemented with 50 ppm of YE and the indicated ^{14}C-labelled substrate. The control values represent the background radioactivity obtained from uninoculated serum bottles.

[b] *Pseudomonas* sp. strains BI7 and BI8 were grown in MSM supplemented with the indicated *n*-alkane as the sole carbon source. Bacterial growth was monitored by CO_2 respiration. Each value represents the mean amount of CO_2 evolved from triplicate samples, and the standard deviation is shown in parentheses.

viously described (26). Both BI7 and BI8 readily mineralized naphthalene and relatively low levels of dodecane, toluene, and hexadecane at 25°C but only negligible amounts of benzene and fluorene compared to uninoculated controls (Table 1). BI9 mineralized benzene, naphthalene, and toluene but not dodecane, hexadecane, or fluorene (data not shown). The psychrotrophs also mineralized the above substrates at 5°C. The production of CO_2 during growth on C_5 to C_{16} *n*-alkanes as the sole carbon source was determined to verify alkane utilization by *Pseudomonas* sp. strains BI7 and BI8 and to determine the range of alkanes assimilated by these two strains. Serum bottles (100 ml) containing 20 ml of MSM (11) supplemented with 100 ppm of specific alkanes were inoculated with either BI7 or BI8 to a final optical density at 600 nm (OD_{600}) of 0.025, sealed with Teflon septa and crimp caps, and incubated at 25°C with shaking (150 rpm). The serum bottles contained a 5-ml test tube containing 1 ml of 0.1 N KOH to trap CO_2, which was quantified as described by Anderson et al. (1) and reported as micromoles of CO_2 evolved. Uninoculated controls and bottles inoculated with BI7 or BI8 but not supplemented with an *n*-alkane were monitored for background CO_2 evolution. The highest levels of CO_2 were produced by both strains during growth on the shorter-chain alkanes (C_5, C_6, C_7, and C_8) as compared to growth on the longer-chain alkanes (C_{10}, C_{11}, C_{12}, and C_{16}) (Table 1), except for CO_2 evolution by BI8 during growth on C_{11} alkanes. The mineralization data indicate that BI7 and BI8 assimilate C_5 to C_{12} *n*-alkanes, similar to *P. oleovorans*, although *P. oleovorans* mineralizes dodecane to a greater extent (40%) than do BI7 and BI8 (data not shown).

Biodegradation of a mixture of naphthalene, octane, and toluene by *Pseudomonas* sp. strains BI7 and BI8. The ability of *Pseudomonas* sp. strains BI7 and BI8 to degrade a mixture of toluene, octane, and naphthalene in MSM at 25 and 5°C was examined. Serum bottles (100 ml) containing 20 ml of MSM, supplemented with 10 ppm of YE and ~25 to 30 ppm each of toluene, octane, and naphthalene, were inoculated with *Pseudomonas* sp. strain BI7 or BI8 to a final OD_{600} of 0.025. Parallel serum bottles, which were not inoculated with *Pseudomonas* sp. strain BI7 or BI8, were set up as controls to monitor background loss of the three hydrocarbons due to volatilization and/or adsorption. The serum bottles were sealed and incubated at 25 or 5°C as described above. At regular intervals, triplicate samples from both the inoculated and uninoculated control bottles were sacrificed for chemical extraction as follows. An extraction standard, 1,2,3,4-tetrachlorobenzene, was added to a final concentration of 25 ppm. The hydrocarbons were extracted by the addition, through the Teflon septum, of 10 ml of dichloromethane. The serum bottles were vigorously shaken for 10 min on a wrist action shaker (Burrel Scientific), the phases were allowed to separate, and 1 ml of the solvent phase was recovered. The three substrates were quantified by gas chromatography-mass spectrometry (GC-MS) (Saturn 4D ion trap GC-MS instrument [Varian Associates, Walnut Creek, Calif.]) by a modification of U.S. Environmental Protection Agency method series 8260A and 8270B. A DB-5 column (15 m by 0.25 mm by 0.25 μm film; J & W Scientific, Folsom, Calif.) with He as the carrier gas and a temperature program of an initial oven temperature of 60°C increased at a rate of 7°C/min to 220°C, followed by 15°C/min to 280°C was used. The mass spectra were obtained with an electron impact of 70 eV and a mass range of 30 to 400 amu. For an injection standard, dichlorobenzene was added to a final concentration of 20 ppm to the 1-ml extraction samples before injection. A preliminary investigation, involving 0.02% (wt/vol) sodium azide-killed suspensions of *Pseudomonas* sp. strains BI7 and BI8 (OD_{600} = 0.2) in MSM plus the contaminants showed that depletions of the three petroleum hydrocarbon substrates as a result of adsorption of these compounds to BI7 or BI8 cellular material or the container were negligible (~2% for naphthalene and toluene, ~5% for octane). Both BI7 and BI8 preferentially degraded naphthalene, followed by octane and toluene, as determined by both the rate and extent of biodegradation of the three compounds (Fig. 1). At 25°C, both organisms immediately degraded naphthalene until this compound was undetectable (after 1 to 2 days). In comparison, appreciable utilization of octane by BI7 and BI8 was observed after 2 to 3 days of incubation at 25°C, and small amounts were still detectable after 8 days. Utilization of toluene by BI8 was similar to that of octane, except that greater amounts of toluene were still present after 8 days at 25°C. BI7 did not utilize significant amounts of toluene at 25°C in the presence of naphthalene and octane. Except for longer lag phases observed at 5°C, the pattern of substrate utilization by BI8 at the lower temperature was similar to that observed at 25°C. BI7 appeared to assimilate octane and toluene to a greater extent at 5°C than at 25°C, indicating that this organism may be physiologically better adapted for assimilation of these substrates at lower temperatures. The preferential utilization of naphthalene by BI8 at 5 and 25°C and BI7 at 25°C, coinciding with the apparent lag phases observed for octane and toluene utilization, suggests that sequential growth on the three substrates may be occurring.

PCR and sequence analyses of BI7, BI8, and BI9. The psychrotrophic strains BI7, BI8, and BI9 were screened by PCR for the presence of catabolic genes in known bacterial biodegradative pathways for C_5 to C_{12} *n*-alkanes (*alkB*), naphthalene (*ndoB*/*nahAc*), and toluene (*xylE*) by using oligonucleotide primers derived from these genes (26). The following refer-

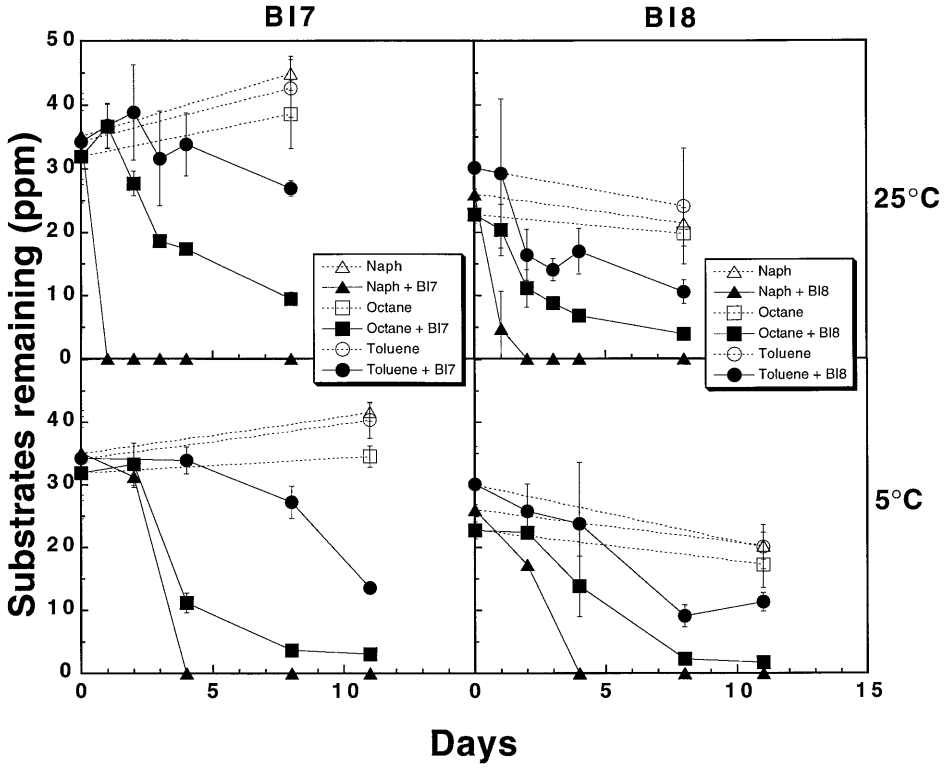

FIG. 1. Biodegradation of a mixture of naphthalene (Naph), octane, and toluene by *Pseudomonas* sp. strains BI7 and BI8 at 25 and 5°C. The cells were incubated in MSM supplemented with 10 ppm of YE and ~25 to 30 ppm of each of the three hydrocarbon substrates. The three hydrocarbons were extracted with dichloromethane and quantified by GC-MS analysis. Each point represents the mean ± standard deviation of extractions from triplicate cultures.

ence strains, cultured at room temperature on TSA and stored at 4°C, were used as positive controls: *Pseudomonas putida* mt-2 ATCC 33015 (*xylE*$^+$), *P. oleovorans* ATCC 29347 (*alkB*$^+$), and *P. putida* ATCC 17484 (*ndoB*$^+$). To verify PCR amplification, PCR fragments the same size as the positive controls were cut out of the agarose gels and purified with the QIAEX II gel extraction kit (Qiagen, Chatsworth, Calif.). The purified PCR fragments were sequenced with the ABI PRISM dye terminator cycle-sequencing ready reaction kit (Perkin-Elmer, Montreal, Quebec, Canada) and the 373A automated fluorescence sequencer (Applied Biosystems, Foster City, California) as specified by the manufacturers. DNA sequences homologous to the nucleotide sequences generated from the PCR fragments were searched for in the GenBank databases with the BLASTN and BLASTX programs. DNA and amino acid sequences with the greatest homology to the sequences obtained from the PCR fragments were analyzed with GeneWorks II software (IntelliGenetics, Mountain View, Calif.).

PCR analysis of BI7 and BI8 resulted in the amplification of both *alkB* and *ndoB* DNA fragments of similar sizes to the control strains (Fig. 2). Nucleotide sequence analysis of the BI7 and BI8 *alkB* PCR fragments revealed that they had 84 and 82% nucleotide sequence identity, respectively, to the *P. oleovorans alkB*. The derived amino acid sequences of BI7 and BI8 AlkB had 94 and 92% similarity, respectively, to *P. oleovorans* AlkB. BI7 and BI8 AlkB were 96% similar at the amino acid level. The BI7 *ndoB* PCR fragment had 99% DNA sequence identity to *nahAc*, and the amino acid sequence had 100% similarity to that of NahAc (20). The BI8 and BI9 *ndoB* PCR fragments had 100 and 99% DNA sequence identities to *ndoB*, respectively, while the derived amino acid sequences of BI8 and BI9 NdoB had 100 and 99% similarities, respectively, to that of NdoB (14).

The BI7, BI8, and BI9 *xylE* PCR fragments (data not shown) had 98, 88, and 94% DNA sequence identities, respectively, to *nahH* (the *xylE* homolog [81% DNA sequence identity] of the *nah* pathway of *P. putida* G7 [9]), while their derived amino acid sequences had 98, 88, and 89% amino acid similarities, respectively, to that of NahH. Thus, the oligonucleotide primers designed to amplify *P. putida* ATCC 33105 *xylE* will also amplify *xylE* homologous genes such as *nahH*. Subsequently, a *xylE* PCR fragment obtained from *P. putida* ATCC 17484 was sequenced. A GenBank database search revealed that it

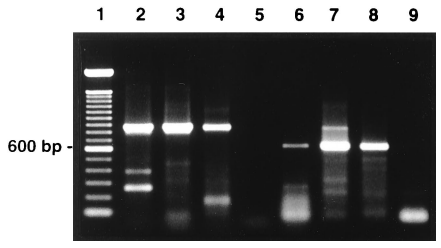

FIG. 2. Detection of *alkB* and *nahAc*/*ndoB* in *Pseudomonas* sp. strains BI7 and BI8 by PCR analysis with oligonucleotide primers specific for *alkB* and *nahAc*/*ndoB*. The samples were subjected to agarose gel electrophoresis (1.2% agarose). Lanes: 1, 100-bp ladder; 2, *P. oleovorans* ATCC 29347 (*alkB*-positive control); 3, *Pseudomonas* sp. strain BI7 (*alkB*); 4, *Pseudomonas* sp. strain BI8 (*alkB*); 5, H_2O (*alkB*-negative control); 6, *P. putida* ATCC 17484 (*ndoB*-positive control); 7, *Pseudomonas* sp. strain BI7 (*nahAc*/*ndoB*); 8, *Pseudomonas* sp. strain BI8 (*nahAc*/*ndoB*); 9, H_2O (*nahAc*/*ndoB*-negative control).

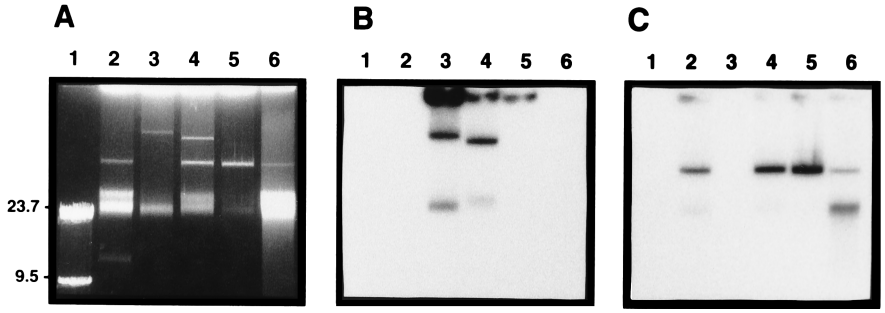

FIG. 3. Plasmid analysis and localization of *alkB* and *nahAc*/*ndoB* in *Pseudomonas* sp. strains BI7 and BI8 by Southern analysis. Total DNA (chromosomal and plasmid DNA) from psychrotrophic biodegradative *Pseudomonas* sp. strains BI7, BI8, and BI9 was isolated and examined by gel electrophoresis and Southern analysis for the localization of *alkB* and *nahAc*/*ndoB*. (A) Agarose gel electrophoresis (0.7% agarose) showing the presence of large plasmids in the psychrotrophic isolates. (B) Southern hybridization analysis of chromosomal and plasmid DNA shown in panel A transferred to a nylon membrane and probed with the 870-bp *alkB* gene probe derived from *P. oleovorans* ATCC 29347. (C) Southern hybridization analysis of chromosomal and plasmid DNA shown in panel A transferred to a nylon membrane and probed with the 642-bp *ndoB* gene probe derived from *P. putida* ATCC 17484. Lanes: 1, lambda (*Hin*dIII) ladder; 2, *P. putida* ATCC 17484 (NAH3 plasmid); 3, *P. oleovorans* OCT plasmid); 4, *Pseudomonas* sp. strain BI7; 5, *Pseudomonas* sp. strain BI8; 6, *Pseudomonas* sp. strain BI9.

had the greatest DNA sequence identity (90%) to *nahH* and 70% identity to *xylE*. However, further DNA sequence comparisons revealed that *P. putida* ATCC 17484 *xylE* was almost identical to BI8 and BI9 *xylE*, having 98 and 99% DNA sequence identities, respectively. These results suggest that *P. putida* ATCC 17484 *xylE* and BI8 and BI9 *xylE* are most probably the *nahH* from the *nah* pathways found in these organisms.

Based on PCR and DNA sequence analysis, BI7 and BI8 appear to possess *alk* pathways comparable to the *P. oleovorans alk* pathway. The data also suggests that the *nah* pathways in the two bacteria are different; BI7 has *nahH* and *nahAc* genes highly homologous to those found in the archetypal *nah* pathway of *P. putida* G7 (9, 20), while BI8 and BI9 possess *ndoB* genes and probably *nahH* genes homologous to those found in the *nah* pathway of *P. putida* ATCC 17484.

Compared with their mesophilic counterparts, cold-adapted enzymes from bacteria and other ectothermic species usually are more thermolabile and possess higher specific activities at low temperatures as a result of a more flexible protein conformation (6). Along with other structural adaptations, a general decrease in the overall hydrophobicity of cold-adapted proteins can be associated with their increased thermosensitivity and flexibility (6). To determine if BI7 and BI8 AlkB had undergone similar cold adaptations, the deduced AlkB amino acid sequences (representing 269 of 401 amino acids of AlkB) from psychrotrophic BI7 and BI8 were directly compared with the corresponding 269-amino-acid segment of AlkB of mesophilic *P. oleovorans* by using the SEQSEE protein analysis software version 1.2(c) (Protein Engineering Network of Centres of Excellence, University of Alberta). The average hydrophobicity value was 0.50 for *P. oleovorans* AlkB, compared with the more hydrophilic values of −0.27 for BI7 AlkB and −0.18 for BI8 AlkB. This result suggests that the alkane hydroxylase component of the alkane catabolic pathways found in these two psychrotrophic microorganisms may have undergone cold adaptation, allowing these organisms to more effectively degrade alkanes at low temperatures. In comparison, similar analyses of the deduced amino acid sequences of the *nah* genes from BI7, BI8, and BI9 revealed that their average hydrophobicity values were equal to or slightly lower than the corresponding amino acid segments of NahAc and NahH from *P. putida* G7 and NdoB and "NahH" from *P. putida* ATCC 17484, both of which are also psychrotrophic bacteria.

Plasmid isolation and Southern analysis. Psychrotrophic *Pseudomonas* sp. strains BI7, BI8, and BI9 were screened for the presence of large catabolic plasmids, similar to known OCT and NAH plasmids, by the method of Wheatcroft et al. (25). Southern analyses of the plasmid bands found in these organisms, with DNA probes specific for *alkB* and *ndoB*, were performed essentially as described by Whyte et al. (26) with high-stringency hybridization and washing at 65°C. As shown in Fig. 3, BI7, BI8, and BI9 possessed $ndoB^+$ plasmids (i.e., NAH plasmids), which migrated the same distance in the 0.7% agarose gel as the 115-kb NAH3 plasmid found in *P. putida* ATCC 17484. The $ndoB^+$ plasmids and NAH3 also comigrated with the 83-kb NAH7 plasmid of *P. putida* G7 (data not shown), which, as suggested by Conners and Barnsley (3), may be because NAH3 is more highly supercoiled than NAH7. Restriction enzyme (*Bam*HI) analysis of the BI8 and BI9 NAH plasmids, which were individually purified from low-melting-point agarose gels, resulted in *Bam*HI restriction patterns closely resembling those observed (data not shown) and previously reported for NAH3 (3). In addition, Southern analysis of BI8 and BI9 *Bam*HI restriction patterns with an *ndoB* gene probe generated a ~9.6-kb *Bam*HI $ndoB^+$ fragment with a size identical to that observed for NAH3, indicating that the BI8 and BI9 NAH plasmids appear to be closely related to NAH3 and, as shown by the DNA sequence analyses, contain *nah* genes highly homologous to those found on NAH3. Restriction enzyme (*Bam*HI) and Southern (*ndoB*) analyses of the BI7 NAH plasmid generated a restriction pattern similar to that previously reported for NAH7 (3) and a ~6.0-kb *Bam*HI $ndoB^+$ fragment with a size identical to that observed for NAH7 (data not shown). BI7 contained another plasmid, slightly smaller than the 340- to 500-kb OCT plasmid of *P. oleovorans*, which hybridized to the *alkB* gene probe (Fig. 3). As well as the difference in size, the BI7 $alkB^+$ plasmid may not be similar to OCT. As well as specifying alkane degradation, OCT confers mercury resistance in *P. oleovorans* (23), and, unlike *P. oleovorans*, neither BI7 or BI8 was resistant to mercury (as determined by growth on TSA supplemented with 100 μg of $HgCl_2$ per ml). In addition, restriction analysis of the BI7 $alkB^+$ plasmid with *Pst*I and *Eco*RI followed by Southern analysis with an *alkB* gene probe generated different-sized *Pst*I and *Eco*RI $alkB^+$ fragments compared with the 4.6-kb *Pst*I and 16.9-kb *Eco*RI $alkB^+$ fragments generated from OCT (data not shown). BI7 clearly contains both a NAH plasmid and an $alkB^+$ plasmid apparently containing the previously characterized *nah* and *alk* catabolic pathways responsible for the observed naphthalene and alkane degradation by this organism. Despite repeated attempts to find a plasmid in BI8 similar to

OCT or the BI7 *alkB*$^+$ plasmid, by using a number of different plasmid isolation techniques designed to detect large plasmids in bacteria (12, 15, 25, 28), we were not able to conclusively localize the *alkB* gene in BI8.

Although the incompatibility groups of the BI7 NAH and *alkB*$^+$ plasmids were not determined, the high homologies of the BI7 plasmid-encoded *nahAc* and *nahH* genes to their counterparts on NAH7 and the *Bam*HI restriction and Southern analyses of the BI7 NAH plasmid suggest that the BI7 NAH plasmid is closely related to the IncP-9 NAH7 plasmid (19). The BI7 *alkB*$^+$ plasmid could be an IncP-2 plasmid, similar to OCT (19), or another Inc group besides IncP-9. While alkane-catabolic plasmids, such as OCT or the BI7 *alkB*$^+$ plasmid, and NAH plasmids appear to be compatible, it is surprising that naturally occurring microorganisms containing both types of plasmids, similar to BI7, appear to be rare. Perhaps the heavy metabolic load that two such large catabolic plasmids might impose on these bacteria outweighs the competitive advantage given to such an organism in natural ecosystems, except in environments contaminated with both alkanes and PAHs.

The possession by both psychrotrophic organisms of alkane catabolic pathways similar to the *alk* pathway of mesophilic *P. oleovorans* is in itself interesting, suggesting that the transfer of biodegradative pathways between mesophiles and psychrotrophs has occurred or is occurring in nature and that the pathways are expressed in both types of organisms. Evidence supporting this hypothesis was previously obtained by demonstrating the successful transfer by conjugation of the TOL plasmid from a mesophilic *P. putida* to a psychrotrophic *P. putida* in which the toluene biodegradative genes were expressed at 0°C (13). Although the results presented here provide indirect evidence that such transfers can occur in nature, these genetic exchanges are not unexpected because many catabolic pathways are found on transmissible plasmids and, in many cases, the catabolic pathways, including *nah* pathways (27) and perhaps the *alk* pathway (23), are located within transposable elements.

In conclusion, we have shown that psychrotrophic microorganisms that possess both *alk* and *nah* pathways, responsible for the biodegradation of both alkanes and naphthalene, can exist in nature. These psychrotrophic strains readily utilized these contaminants at 5°C, indicating their potential utilization for low-temperature bioremediation of contaminated sites. The similarity of the *Pseudomonas* sp. strain BI7 NAH and *alkB*$^+$ plasmids to NAH7 and OCT are being investigated to better understand the basis of their compatibility and the role they play in both alkane and naphthalene degradation.

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